International Journal of Engineering Science Invention (IJESI)
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org ||Volume 8 Issue 04 Series. I || April 2019 || PP 48-52

Bioremediation ofCongo Red, a synthetic textile azo dye by a halotolerant bacterial strain.

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Abstract:*Due to rapid increase in industrialization and urbanization it results in the emission of waste to the environment, which generates more pollution. The ejection of toxic sewage from different textile industries affects the water resources, fertility of soil, aquatic life and ecosystem integrity. Bioremediation of textile dyes is a method that is mostly used and is of considerable interest since it is not expensive, environmental friendly and generates a small amount of sludge. In the present study, effluent samples were collected from various textile and dyeing industries located in India and were utilized for the screening and isolation of bacterial strains that were capable of decolorizing the textile dye, Congo Red. Physicochemical properties of the effluent samples were examined. Five bacterial strainswere able for decolorizing Congo Red were screened and isolated from various effluent samples. Out of which, Bacillus sp. Strain showed maximum decolourization efficiency of 86.43% within 30 h of incubation. HPLC chromatogram and FTIR spectrum of 24 h extracted metabolites showed notable change in the positions of peaks, when compared to control dye spectrum, indicating the biodegradation of Congo Red.*

Keywords : *Channa channastriata, Congo Red, Phytotoxicity, Sorghum vulgare*

Date of Submission: 29-03-2019 Date of acceptance: 09-04-2019

I. Introduction

Since from the beginning of this era, people are using colorants for painting and dyeing their surroundings and their clothes. Initially the colorant materials were used in walls of Altamira cave in Spain.The pioneering synthesis of mauveine by W. H. Perkins started the era of synthetic dyes, with chemical and physical properties better suited to contemporary demands, better level of quality and more reproducible techniques of application (Clark et al., 1993; Vijayanand and Hemapriya, 2013). Now there are more than 1,00,000 commercially available dyes whilst over 7 x 105 metric tons of dyestuffs are produced annually (Wong and Yu, 1999). Dyes are used in many fields such as textile industry, leather tanning industry, paper and pulp industry, food industry, agricultural research, photo electrochemical cells, hair colouring, cosmetics etc. Moreover, these compounds have been employed for the control of the efficacy of sewage and wastewater treatment, for the determination of specific surface area of activated sludge and for ground water tracing (Forgacs et al., 2004; Hemapriya and Vijayanand, 2013).

Huge quantity of waste water is produced in textile industry during wet processing and this is not only because of eliminating the impurities from raw materials but also from the chemical reagents that were used during the processing. The extreme diversity of raw materials and production schemes employed poses problems in assessing effluent characteristics and subsequently defining pollution control technologies (Correia et al., 1994). Throughout the textile processing, the carelessness in dyeing would result huge amount of dyestuff which would instantly lost to waste water and will eventually enter the environment. The amount of dye lost is dependent upon the dye application class, varying from only 2% loss when using basic dyes to a 50% loss when certain reactive dyes are used (O Neill et al., 1999; McMullan et al., 2001; Pearce et al., 2003). Colour present in the dye effluent gives a straightforward indication of water being polluted (Nigam et al., 1996). It is aesthetically displeasing, as it hinders the penetration of sunlight, and this indirectly affect photosynthesis and some biological treatment systems which are algal based such as aerated lagoons etc.

As dyes decrease the penetration of light it may crucially affect photosynthetic activity of aquatic life and due to presence of aromatic substances or heavy metals it may be toxic. Large amount of work has been done on the pollution problems related with the release of textile discharge. Several psychological and biochemical disturbances occur in fish if there is any change in quality of water. From thedye discharge the toxic compounds that are present in them enter aquatic organisms, and indirectly enter the food chain and eventually reach human beings and this leads to numerous physiological disorders and many diseases like hypertension, renal damage, cramps, etc. Bioaccumulation of toxicants depends on the availability and

persistence of the contaminants in water, food and physico-chemical properties of the toxicants (Puvaneswari et al., 2006).

Due to chemical stability of these pollutant present in textile dyes it has been proved distinctly that conventional wastewater treatment mechanisms is being unsuccessful for handling wastewater of synthetic textile dyes. For removal of the synthetic dyes from wastewater or water to reduce their effect on environment and life many methods have been developed.

According to Kalyani et al. (2009), Bioremediation of textile effluents has been of considerable significance since it is not very expensive, as it is not harmful to environment and it does not produce a more quantity of sludge. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actinomycetes, fungi, yeasts and algae capable of degrading azo dyes (Chen et al., 2003; Daneshwar et al., 2007). Taking into account all the advantages and applications of bioremediation process in treating the wastewater, the present research is done on bioremediation of Congo Red, this is a synthetic textile azo dye by a moderately halo-tolerant bacterial strain.

II. Methods

Sampling Site, Sample Collection and Physico-chemical analysis

The sampling site was the textile industries and dyeing units that was located in India. The effluent samples from both textile industries and dyeing units were distinguished by its dark colour and utmost turbidity. Samples were taken from the surface and from differentdepths and were put in sterile polythene bags to stop their direct contact with air and taken to the laboratory in an ice box for additional analysis. Physico-chemical properties of the effluent samples were analysed (APHA, 1980).

Synthetic Azo Dye Used

The often used textile azo dye, Congo Red dye was used in this study was obtained from a local textile dyeing unit. Stock solution was prepared by dissolving 0.5 g of Congo Red in 100 ml distilled water. The dye solution was sterilized by membrane filtration (Millipore Millex ® - GS, 0.22 Mm filter unit), since azo dyes may be changeable to moist-heat sterilization. All the chemicals used in this study were of the highest purity available and of an analytical grade.

Toxicity studies with Channa channastriata

Channa channastriata used in this study was taken and incubated in a container containing 1 L of water with 110 ppm of Congo Red and the control was setup by transferring and incubating another fish of same species, size and age in a container containing 1 L of water (without any dye products). Favourable conditions such as pH 7.0, proper aeration and room temperature was provided for 42 h. Following incubation, the fishes were killed (figure not shown) and the histopathology studies were carried out with the gills and skin of fish samples (control and test).

Isolation and Screening of Bacterial Strains Decolorizing Congo Red

The effluent samples were serially diluted and incubated over basal nutrient agar medium containing 60 ppm of Congo Red at 37°C for 6 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on nutrient agar plates containing azo dyes (Wong and Yuen, 1996). Separate colonies of dye decolorizing bacteria were picked and restreaked many times to get pure cultures. Decolorization degree of the isolates were resultedby measuring the absorbance of the culture supernatant at 490 nm using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya et al. (2010).

HPLC Analysis of Decolorized Sample

12 ml of decolorized samples were taken after 28 h of incubation, centrifuged at 14,000 g for 45 min, and filtered through 0.45 µm membrane filter (Millipore). The filtrates were then separated with diethyl ether and flash evaporated in rotary vacuum evaporator in temperature controlled water bath (50°C) and residues were dissolved in 4 ml of HPLC grade methanol and used for analysis. These extracted samples were analysed by HPLC having a mobile phase of 55:50:0.5% (methanol: water: disodium hydrogen phosphate).

FTIR Analysis of Decolorized Samples

The biodegraded azo dye samples were characterized by FTIR spectroscopy (PerkinElmer, Spectrum one). The analysis results were compared with the control dye. The FTIR analysis was done in the mid IR region (400-4000 cm-1) with 16 scan speed. The samples were mixed with spectroscopically pure KBr in the ratio (6:95). The pellets were fixed in sample holder and then analysed (Saratale et al., 2009).

Phytotoxicity Studies

Phytotoxicity tests were executed in order to estimate the toxicity of the untreated and treated dye samples. The ethyl acetate separated products of degraded azo dyes were dried and dissolved in 10 ml sterile distilled water to make a final concentration of 100 ppm. The Phytotoxicity tests were carried out on Sorghum vulgare Pers. (monocot) (Parshetti et al., 2006). 12 healthy plant seeds were treated separately with 7 ml of control dye and degraded products respectively/per day. Control sets were carried out using distilled water at the same time. Germination percentage as well as the length of plumule and radical was recorded after 10 days (Saratale et al., 2009).

III. Results and Discussion

In modern world one of the major problems that has been identified is environmental pollution. Due to increase in population the need for water and other necessary thing is also increasing and the supply of the needs is being reduced and this made an attractive option to treat and reuse the industrial effluent. Wastewater from textile and dyestuff industry is a worldwide problem because they have the colour and they colour the drains and eventually colour the water bodies. Considerable amount of dyes and many other chemicals are lost in discharge during the dyeing process. Moreover, these azo dyes and their intermediate aromatic amines are either toxic or mutagenic or carcinogenic, posing a potential health hazard to human kind (Carliell et al., 1995). So,to treat and to make clean the textile effluents has become important and is matter of great concern. One promising strategy is the use of microbial strains that possess the ability to decolorize and mineralize synthetic dyes (Robinson et al., 2001).

Physico-Chemical Analysis of Effluent Samples

The average temperature at the sampling sites was around 38°C at day time. The physico-chemical characteristics of the effluent samples were shown in the Table.1. The pH values of the effluent samples were found to be alkaline. Total solids of T2 and T3 samples were found to be lower than the T1 sample. The highest TSS content was encountered in T3 sample. TDS content was almost same in both T1 and T3 samples. BOD value of T1 sample was found to be higher than the T2 and T3 samples. However, the COD value was maximum in case of T2 sample. The effluent samples collected from T1, T2 and T3 sites were found to be dark blue, blackish blue and dark brown respectively.

Dye Stuff Used

The dye stuff used in this study was Congo Red with molecular formula of $C_{23}H_{26}N_2O.HCl$. The absorption maximum of this dye was 490 nm. The structure of Congo Red was shown in Fig.1.

Toxicity Analysis of Congo Red in Channa channastriata

Results of histopathological studies showed marked changes in the sections of gills and skin of Murrel fishes (control and test). The fish exposed to dye showed the clubbing of lamellae, vascular degradation, lifting of primary lamella and high level proliferation of epithelial cell. The blood vessels were not properly circulated in the fish. Tubular damage distinguished by vacuolated, degenerated, hypertrophied tubular epithelial cells and occlusion of tubular lumen was recorded at all alterations (Figure not shown).

However, no notable changes were observed in the histological sections of skin samples (Fig not shown). Similarly, exposure of fish, Oreochromis mossambicus to sub-lethal concentration of effluent strongly affected the rates of feeding, absorption and conversion. Protein contents of muscle, liver, gill and intestine decreases with increasing concentrations of dye effluent (Amutha et al., 2002).

Isolation and Screening of Bacterial Strains Decolorizing Congo Red.

The results shown in Table.2 revealed that five isolates designated as C1 to C-5 were found to be effective in decolorizing Congo Red. Out of which, C3 exhibited the highest decolorization efficiency of about 85.23 %.

Based on the Morphological, cultural, biochemical characteristics and 16s r DNA analysis, C3 isolate was identified as a moderate halotolerant bacterium - Bacillus sp C3. Similarly, Bacillus gordonae and B. thuringiensis exhibited excellent decolorization of Tectilon Blue 4R-01 and Acid Red-119 respectively (Walker and Weatherley, 2000; Dave and Dave, 2009).

HPLC Analysis of Decolorized Sample

To explain the exact occurrence of dye decolorization, the HPLC analysis of dye sample was carried out at 0 h incubation that showed the presence of 1 major peak with retention time of 9.707 min (data not shown). As the decolorization progressed, the biodegradation of parental dye compound was observed with 23 detectable peaks at 20 h extracted metabolites, however major peak was not observed at 9.707min, clearly

indicating the biodegradation of Congo Red by Bacillus sp. Strain C3. This result was in complete accordance with the findings of Kalyani et al. (2009).

FTIR Analysis of Decolorized Sample

Comparison of FTIR spectrum of the control dye with extracted metabolites after complete decolorization clearly indicated the biodegradation of Congo Red by Bacillus sp. strain C3 (Data not shown). The results of FT-IR analysis of Congo Red parent dye and sample obtained after decolorization showed various peaks. The FT-IR spectra of Congo Red parent dye displayed peaks at 3477, 2915, 1570 and 1427 cm-1, for OH stretching vibration, aromatic -CH stretching vibration, -C=C- stretching and -N=N- stretching vibration respectively. However, the FT-IR spectra of degradation product displayed peaks at different positions indicating the complete breakdown of Congo Red.

Phytotoxicity Assay

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated Acid Orange-10 dye samples (Fig not shown). S. vulgare seeds treated with tap water showed 100% germination, the mean plumule length of 27.30±1.10 cm and the mean radical length of 6.20±1.00 cm.

Fig.1 Chemical Structure of Congo Red

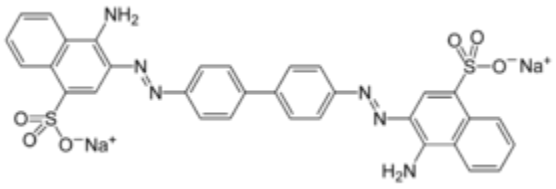

Table 1 Physio chemical characteristics of tannery effluent.

Parameter	T1	T2	T3	Permissible limit
pH	9.0	8.4	7.9	6.0-8.0
Colour	Dark Blue	Blackish Blue	Dark Brown	850
TS (mg/ L)	2,300	2,500	2,620	2,200
TDS (mg/ L)	2,200	2,740	2,140	2,100
TSS (mg/ L)	279	295	370	100
DO (mg/ L)	2.6	3.4	3.2	4.0-6.0
BOD (mg/ L)	245	145	169	30
COD (mg/ L)	365	447	330	250
Sulphate (mg/ L)	2,285	2,215	2,265	1,000
Magnesium (mg/ L)	255	225	315	200
Phosphate (mg/ L)	5.7	6.5	4.5	5.0
Nitrate (mg/ L)	11.65	12.5	10.86	10
Fluoride (mg/ L)	4.2	3.6	2.5	2.0
Phenol (mg/ L)	6.2	5.9	4.5	1.0
Oil and grease	15.5	14.5	16.5	10

Table.2 Bacterial Strains Decolorizing Congo Red (C1 to C5)

SR. NO.	Isolates	Sample Collection Site	% of Decolorization
1	C1	T2	70.98 %
2	C2	T3	65.15 %
3	C3	T2	88.48 %
4	C4	T1	62.44 %
5	C5	T1	64.18 %

Note: The isolates are considered for the table only with 50% decolorization ability.

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